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Signed Andrew Gerrey

Dated 9 October 2003

Patents Form 1/77

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# Request for grant of a patent

*(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)*

The Patent Office
Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference — RJS/JN/B45323

2. Patent application number
*(The Patent Office will fill in his part)* — 23 OCT 2002 — 0224688.2

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

GlaxoSmithKline Biologicals s.a.
Rue de l'Institut 89, B-1330 Rixensart, , Belgium

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — Belgian

8101271001

4. Title of the invention — Method

5. Name of your agent *(if you have one)* — Corporate Intellectual Property

"Address for service" in the United Kingdom to which all correspondence should be sent
*(including the postcode)*

GlaxoSmithKline
Corporate Intellectual Property (CN9 25.1)
980 Great West Road
BRENTFORD
Middlesex TW8 9GS

Patents ADP number *(if you know it)* — 07960972005

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or each of these earlier applications and *(if you know it)* the or each application number

| Country | Priority application number *(if you know it)* | Date of filing *(day / month / year)* |
|---|---|---|

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

| Number of earlier application | Date of filing *(day / month / year)* |
|---|---|

8. Is a statement of inventorship and of right to grant of a patent required in support of

this request? *(Answer yes if:*

*a) any applicant named in part 3 is not an inventor, or*

*b) there is an inventor who is named as an applicant, or*

*c) any named applicant is a corporate body*

*See note (d)*

*attached to this form.*

*d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.*

*f) For details of the fee and ways to pay please contact the Patent Office.*

**Patents Form 1/77**

Corporate Intellectual Property
GlaxoSmithKline, CN925.1, 980 Great West Road, Brentford, Middlesex TW8 9GS. Tel: 020 8047 5000 Fax: 020 80476894

antigen and a basic buffer.

**Detailed description**

The present invention is based generally on the ELISA detection methods, in which the binding of an antigen to an antibody to in the context of a solid support is then detected by the binding of a second antibody. ELISA methods are well known in the art (see, for example, Belanger *et al.* Clin.Chim. Acta 48, pages 15-18).

The method of the invention relates to the detection of an antigen in combination with aluminium hydroxide. Preferably an antigen which is in combination with aluminium hydroxide is adsorbed or otherwise directly complexed or associated with aluminium hydroxide. The invention, however, also relates to the detection of an antigen which is not itself directly adsorbed or complexed to aluminium hydroxide, but is in a mixture or composition in which aluminium hydroxide is also present. The aluminium hydroxide may be free or bound to an antigen which is not the same as the antigen to be detected by the assay.

Preferably the antigen is a hepatitis antigen, preferably a hepatitis B antigen, most preferably hepatitis B surface antigen.

Preferably the method of the invention is for the detection and/or quantification of a hepatitis B surface antigen adsorbed onto aluminium hydroxide.

The invention also relates to detection and/or quantifiation of a hepatitis B antigen adsorbed or associated with an aluminium salt, preferably aluminium phosphate, in a combination with another antigen adsorbed or associated with aluminium hydroxide. In particular the detection may be of a hepatitis B antigen in a combination of hepatitis B surface antigen adsorbed on aluminium phosphate with pertactin adsorbed onto aluminium hydroxide. In such a case it will be appreciated that the invention applies also to the detection of the pertactin component.

In the first stage of the process an antigen is contacted with an immunoglobulin or fragment thereof in the context of a solid support. The contacting of antigen with immunoglobulin suitably occurs when one or other is bound to an appropriate solid support. Preferably the immunoglobulin or fragment thereof is bound to the solid support. The solid support is preferably a plastics solid support, suitably a microtitre plate or other plate appropriate for an ELISA-type analysis.

Preferably the immunoglobulin or fragment thereof is affixed to the solid support and then this fixed immunoglobulin or fragment thereof is contacted with the antigen.

Most preferably a plastics microtitre plate is coated with a suitable immunoglobulin according to well known methods in the art.

Preferably the immunoglobulin component is an antibody or fragment thereof capable of specific binding to the antigen. Suitable fragments of antibodies which retain specific binding activity for a given antigen are well known in the art and may include antibody Fv regions in the absence of Fc regions or may include suitable single chain immunoglobulins. The term 'antibody' will be used herein to describe all suitable immunoglobulins and fragments thereof which have suitable specific binding for an antigen to allow their use in an ELISA or ELISA type detection system. Preferably the antibody is a polyclonal antibody, most preferably a rabbit polyclonal antibody, with a rabbit antibody against hepatitis B most preferred.

The production and characterisation of antibodies for the detection of hepatitis B surface antigen is well known, for example, as described in Wands *et al*, gastroenterology 80, 225 – 232, 1981 and Shih JW-K *et al* J Virol methods, 1, 257 – 273, 1983.

The mixing of the antigen sample with the antibody is carried out in the presence of a basic buffer, which may be any suitable buffer having a pH greater than 7. Preferably the buffer has a pH of greater than 8, more preferably having a pH of greater than 8.5 and

most preferably having a pH of substantially 9. Preferably the pH is between 7 and 12, more preferably between 8 and 11, most preferably between 8 and 10. The pH can be adjusted to take account of the specific antigen being tested, to optimise the method –that is, to optimise binding and/or minimise the effect of aluminium hydroxide on the assay. Preferably the buffer contains 1% Tween or functional equivalent thereof. Most preferably the buffer is DEA 0.2M, HCl 0.2M at pH9 with 1% Tween added, preferably for use in the detection of hepatitis B surface antigen.

Suitably the antigen to be tested is mixed or diluted into a basic buffer and then contacted with an antibody affixed to a solid support.

Preferably the incubation of the antigen and antibody is carried out with agitation.

Antigen –antibody binding is followed by treatment of the antigen-antibody combination on the solid support with a blocking agent. The blocking agent is any suitable agent that minimises non-specific interactions between the antibody- antigen complex and any detection system used to detect the antibody – antigen complex on the solid support. Suitable blocking agents are well known in the art, with a preferred blocking agent being PBS 1% BSA.

The detection of the antigen-antibody combination may be carried out using any suitable detection means, and these are well known in the art for the ELISA method. An example of a suitable method is exemplified in Example 1.

Preferably the antibody- antigen combination, after blocking, is contacted with a second antibody which binds specifically to the antigen. The binding of the second antibody may be detected directly or indirectly, suitably in order to assess the quantity of antigen present in the sample.. For example, the second antibody may be directly linked to a detectable label or may be detectable by addition of a further labelled antibody. Both direct and indirect detection methods are well known in the art.

Suitable for use in detection is the RF-1 monoclonal antibody (Goodhall AH *et al.*1981, Medical Laboratory Sciences 38, 349-354), preferably in combination with other monoclonal antibodies.

Preferably the detection of the antibody-antigen complex is carried out in a buffer with a relatively high concentration of protein, such as BSA, to again minimise non-specific interactions. Preferably the detection is carried out in a buffer with at least 0.05% blocking protein, such as BSA, more preferably between 0.05% - 0.5% blocking protein, more preferably between 0.1 – 0.3% blocking protein and most preferably approximately 0.2% blocking protein. Most preferably the detection buffer comprises PBS, 0.2% BSA, 0.1% Tween and 4% newborn calf serum, suitably for use in an assay for hepatitis B surface antigen.

In a most preferred embodiment the present invention relates to a method for the detection of a hepatitis B surface antigen in a sample, the hepatitis antigen being adsorbed onto aluminium hydroxide, the method comprising the steps, in order, of:

1 contacting an antibody specific for hepatitis B surface antigen with a sample to be tested, the antibody being bound to a solid support, the contacting being carried out in the presence of a basic buffer, with agitation, to allow binding of the antigen to the antibody, wherein the buffer has a pH of 9 or approximately pH 9;

2 adding a blocking agent comprising 1% BSA or approximately 1% BSA; and

3 detecting the binding of antibody to antigen.

The invention further relates to a kit for use in the detection method described above, the kit comprising instructions for implementing the method as described above and at least one component selected from: a basic buffer suitable for use in the method and an antibody suitable for detection of an antigen of interest. Suitable kits also comprise both antibody and basic buffer, most preferably with instructions. Other kit components to work the method, such as detection buffer, can also optionally be included.

The method and kit of the present invention are suitably used for quality control purposes for vaccine production. In addition, the method and kit may be generally used for antigen identification, measurement of antigenicity and quantification.

The present invention is hereby illustrated by the following example that is not binding upon the present invention.

**Example 1 – Detection of hepatitis B Surface antigen adsorbed onto Aluminium hydroxide in a sample**

1 Microtiter plates were coated with an anti-HBs rabbit polyclonal antiserum.

2 HBs/$Al(OH)_3$ samples to be quantified were diluted in DEA 0.2M, HCl 0.2M 1% Tween pH9 (two-fold dilution) and incubated with the plates for 2 hours at 37°C with agitation.

3 After a washing step (in 150 mM NaCl, 0.05% Tween) the solid phase was blocked with PBS 1% BSA buffer for 1 hour at 37C, then washed.

4 The detection of anti-HBs/HBsAg complex was then performed by addition of a pool of 3 anti-HBs mouse monoclonal antibodies diluted at 1µg/ml in PBS, 0.2% BSA, 0.1% Tween and 4% newborn calf serum and then incubated for 1 hour at 37°C. Excess antibodies were removed by washing and then plates were incubated for 30 min at room temperature (RT) with agitation with a biotin-conjugated anti-mouse Ig (from Prosan™). After washing, the Amdex ™ streptavidin horseradish peroxydase complex (from Amersham™) was added to the wells (30min at RT with agitation) . Plates were then washed and incubated for 20 min with agitation with a solution of o-phenylenediamine (Sigma™) 0.04%, $H_2O_2$ 0.03%, 0.1% tween 20, 0.05M citrate buffer pH4.5. The reaction was stopped with $H_2SO_4$ 2N and read at 490/630 nm. HBsAg concentrations in samples were calculated from a reference by SoftmaxPro and expressed in µg/ml..

The above steps are individually and separately preferred in the present invention for the detection of hepatitis B surface antigen.

**Claims**

1 A method for the detection of an antigen, the antigen being in a combination with aluminium hydroxide, the method comprising the steps of:

i contacting an antigen with an immunoglobulin, or fragment thereof, in the context of a solid support and in the presence of a basic buffer, to allow binding of the antigen to immunoglobulin or fragment thereof;

ii adding a blocking agent; and

iii detecting the binding of antibody to antigen,

wherein steps 1, 2 and 3 are carried out sequentially but not necessarily consecutively.

2 A method according to claim 1 wherein the antigen is hepatitis B surface antigen.

3 A method according to claim 1 or 2 wherein step 1 is carried out with agitation.

4 A method according to any preceding claim wherein the detection step iii is carried out in the presence of 0.2 % BSA.

5 A kit for the detection of an antigen in combination with aluminium hydroxide, the kit comprising instructions for implementing the method of claims 1-4 and at least one component selected from: an antibody specific for the antigen and a basic buffer.

6 A kit according to claim 5 comprising instructions for implementing the method of any of claims 1 to 4, an antibody specific for the antigen and a basic buffer.

7 A method or kit according to any of claims 1-7, wherein the basic buffer is pH 9.